AMENDMENT TO EX PARTE QUAYLE ACTION AND STATEMENT IN RESPONSE TO SEQUENCE LISTING REQUIREMENTS		
	Application #	09/494,297
	Confirmation #	3244
	Filing Date	January 31, 2000
	First Inventor	PODBIELSKI
	Art Unit	1645
	Examiner	Minnifield
	Docket #	P06628US0/BAS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

S I R:

In response to the *Ex Parte Quayle* Office Action dated August 28, 2003 2003, please amend the above identified application as follows.

Please insert the attached Sequence Listing starting at Page 56 of the specification and substitute this sequence listing for any prior sequence listing in the application. **Applicants herein state that the attached sequence listing is identical to the computer readable form attached hereto and that the sequence listing adds no new matter to the application.**

Amendments to the Specification are reflected in the replacement paragraphs provided herewith in **Attachment A**.

Remarks to this Amendment are provided herewith in **Attachment B**.

In light of the above amendments and remarks included herein, the present application has now been placed in condition for allowance.

Respectfully submitted,
LARSON & TAYLOR, PLC

November 26, 2003

B. Aaron Schulman
Registration No. 31877

1199 North Fairfax Street, Suite 900
Alexandria, Virginia 22314
(703) 739-4900

ATTACHMENT A

Amended Paragraphs

At the following locations, please insert the following amended paragraphs.

Please amend the paragraph at page 6, lines 9-20 as follows.

Figure 1 is a schematic representation of a comparison of the *nra (SEQ ID NO:5)/rofA*-associated portions of group A streptococcal serotype M1, M6 and M49 strains. Results of pairwise comparisons of the deduced amino acid sequences of single ORF's are shown as percentage identity values between corresponding sequences. Sequence alignments were centered at the *nra (SEQ ID NO:5)/rofA* to *prtF/cpa* intergenic regions. All sequences are shown to scale. For designation of ORF's, see Table 1 hereinbelow. The M1 sequence was obtained from the GAS sequencing project (Roe et al., 1997), and the M6 sequence was taken from Hanski et al. (1992) and Fogg et al. (1994). The inserted box contains the comparison of the deduced Nra and RofA amino acid sequences. "." marks identical amino acid positions; "-" marks gaps that were introduced into the RofA sequence to maximize alignment. The underlined sequence marks the potential helix-turn-helix identified by Fogg et al. (1997).

Please amend the paragraph at page 45, lines 13-21 as follows.

Plasmid pFW11 was used for insertional mutagenesis as described by Podbielski *et al.* (1996c). Plasmid pFW11 multiple cloning site (MCS) 1. The

luciferase (*luc*) box was amplified by PCR using plasmid pUSL2/5 (Gräfe *et al., 1996)* as template and oligonucleotides lucFor (5'GACGATCTCGAGGAGGTAAATGAAGACGCCAAAAAC-3') (SEQ ID NO:31) and lucRev (5'GACGATAAGCTTTTACAATTTGGACTTTCCG-3') (SEQ ID NO:32) as primers. The luciferase box contained an optimized Shine-Dalgarno sequence as well as the *luc* start and stop codons. Cloning of GAS genomic fragments into MCS1 of pFW11-luc followed the protocol outlined by Podbielski *et al.* (1996c).

Please delete Table 4 at page 43 and insert new Table 43 attached.

TABLE 4. List of oligonucleotides used in this work.

Designation	Sequence (5' to 3')	Sequence ID. No.	Position Numbers	Reference
A.				
nra FOR	ATTTTTTCTCATGTTGCTA	SEQ ID NO:6	6474-6492	This study
nra REV	GTTTAGAATGGTTTAATTG	SEQ ID NO:7	7308-7290	This study
rofA FOR	GCCAATAACTGAGGTAGC	SEQ ID NO:8	141-158	Fogg et al. (1994)
rofA REV	GGCTTTTGCTCTTTTAGGT	SEQ ID NO:9	995-977	Fogg et al. (1994)
cpa FOR	AGTTCACAAGTTGTCTACTG	SEQ ID NO:10	3435-3454	This study
cpa REV	AAATAATAGATAGCAAGCTG	SEQ ID NO:11	3727-3708	This study
prtF FOR	ATTAATGCCAGAGTTAGATG	SEQ ID NO:12	1414-1433	Hanski and Caparon (1992)
prtF REV	CGATTCTCTTCCACTTTG	SEQ ID NO:13	2259-2242	Hanski and Caparon (1992)
prtF2 FOR	TACTCTGTTAAAGAAGTAACTG	SEQ ID NO:14	2260-2281	Jaffe et al. (1996)
prtF2 REV	CTCAGAGTCACTTTCTGG	SEQ ID NO:15	3168-3151	Jaffe et al. (1996)
nifR3 FOR	GGATTTTGCCTACTACTTA	SEQ ID NO:16	8443-8461	This study
nifR3 REV	GTGGAATATCTAAAACAGAC	SEQ ID NO:17	9313-9294	This study
B.				
nra-ins FOR	TTTTATTGGAGACTAGAAGTTTA	SEQ ID NO:18	6325-6347	This study
nra-ins REV	AGCAAGCCACTGATTTAC	SEQ ID NO:19	7481-7464	This study
cpa-ins FOR	TGCAAAAGAGGGATAAAAC	SEQ ID NO:20	5932-5914	This study
cpa-ins REV	GAAGCAGTAGACAACTTGTG	SEQ ID NO:21	4707-4726	This study
nraLuc FOR1	TAAACTAAAGTAGCTTAGCA	SEQ ID NO:22	5953-5972	This study
nraLuc FOR5	ATGGAACGTCATCACAAC	SEQ ID NO:23	6688-6705	This study
nraLuc REV1	CAGATACCTAAAAATAAACG	SEQ ID NO:24	7930-7911	This study
cpa-pMAL FOR	GCTGAAGAACAATCAGTACCA	SEQ ID NO:25	5798-5778	This study
cpa-pMAL REV	TTAGTCATTTTTTAACCCTTTACG	SEQ ID NO:26	3705-3728	This study
C.				
RT-nra FOR	CTTTTTACTTATTAAGAGATGA	SEQ ID NO:27	7669-7690	This study
RT-nra REV	CTCGTTTAGAAAATCTTG	SEQ ID NO:28	7886-7869	This study
RT-orf5 FOR	AAAATAATTAAATCAATAGCA	SEQ ID NO:29	8030-8050	This study
RT-orf5 REV	CCACAGAGATAATGTGT	SEQ ID NO:30	8258-8241	This study

Oligonucleotides were used as primers to PCR amplify probes for Southern and Northern blot hybridizations (A), genomic Fragments for cloning into pFW11, pFW11-luc or pMAL-c2 plasmids (B) and primers for RT-PCR to detect nra and orf5-specific transcripts (C). Primer pairs nra-ins FOR/REV, cpa-ins FOR/REV, nraLuc FOR/REV and cpa-pMAL FOR/REV were 5' extended with SphI/SpeI. NheI/BamHI and BAMHI/PstI sites, respectively, to facilitate forced cloning of the resulting PCR products. The nucleotide position numbers refer to the GAS nra genomic sequence as submitted to GenBank or the cited publications.